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     (FILE 'HOME' ENTERED AT 07:45:37 ON 28 DEC 1999)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, LIFESCI, HCAPLUS, NTIS,
     SCISEARCH' ENTERED AT 07:46:23 ON 28 DEC 1999
L1           874 S IDURONATE (2W)SULFATASE?
L2          3414 S L1 OR IDS
L3        138938 S GLYCOSYLAT?
L4         57343 S CHINESE HAMSTER OVAR?
L5        114122 S L4 OR CHO
L6         77284 S L5(W)CELL?
L7            24 S L2 AND L3
L8            24 S L7 AND L7
L9             0 S L7 AND L6
L10            3 S L5 AND L7
L11            3 DUP REM L10 (0 DUPLICATES REMOVED)
L12           11 DUP REM L7 (13 DUPLICATES REMOVED)
L13           11 S RECOMBINANT(W)L2
L14            2 S L13 AND L3
L15            2 DUP REM L14 (0 DUPLICATES REMOVED)
L16            5 DUP REM L13 (6 DUPLICATES REMOVED)
L17            9 S L13 AND FIBROBLAST?
L18            3 S L16 AND FIBROBLAST?
L19            3 DUP REM L18 (0 DUPLICATES REMOVED)
L20           11 S L7 AND FIBROBLAST?
L21            4 DUP REM L20 (7 DUPLICATES REMOVED)
L22            0 S COMPOSITION(P)L7
L23            0 S L7 AND COMPOSIT?
L24            0 S L16 AND COMPOSITION?
L25           88 S L2 AND COMPOSITION?
L26            1 S L25 AND RECOMBINANT
L27            0 S L25 AND L3
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L11 ANSWER 1 OF 3 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-05849 BIOTECHDS
TITLE: Treatment of **iduronate**-2-**sulfatase** deficiency;
human recombinant enzyme preparation by expression in **CHO**-K1, or Lec-1 cell, used for e.g. Hunter disease therapy or gene therapy
AUTHOR: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J;
Clements P R; Hopwood J J
PATENT ASSIGNEE: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J;
Clements P R; Hopwood J J
LOCATION: North Adelaide, South Australia, Australia; Plympton, South
Australia, Australia; Thebarton, South Australia, Australia;
Prospect, South Australia, Australia.
PATENT INFO: US 5728381 17 Mar 1998
APPLICATION INFO: US 1995-484493 7 Jun 1995
PRIORITY INFO: US 1995-484493 7 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-206530 [18]
AB A new method for treating **iduronate**-2-**sulfatase** (**IDS**, EC-3.1.16.13) deficiency, e.g. Hunter disease, involves administering a human recombinant **IDS** that is more highly **glycosylated** than the naturally occurring enzyme. The recombinant **IDS** has better uptake properties and/or a longer half-life in vivo and is therefore more efficacious than naturally **glycosylated IDS**. Also disclosed are a human recombinant **IDS** with a defined 550 amino acid protein sequence and an isolated genomic DNA fragment carrying all or part of the **IDS** gene. Isolation of the genomic clone may enable gene therapy and genetic analysis of **IDS** deficiency diseases. Preferably, the recombinant **IDS** has a mol.wt. value of 70,000-90,000 by SDS-PAGE and is produced in **CHO**-K1 or Lec-1 cells. (53pp)

L11 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-10390 BIOTECHDS
TITLE: **Glycosylation** variants of **iduronate**-2-**sulfatase**;
human recombinant enzyme preparation by vector-mediated
gene transfer and expression in host cell, used for Hunter
syndrome diagnosis or therapy
AUTHOR: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J;
Clements P R; Hopwood J J
PATENT ASSIGNEE: Women's+Child.Hosp.North-Adelaide
LOCATION: North Adelaide, South Australia, Australia.
PATENT INFO: US 5798239 25 Aug 1998
APPLICATION INFO: US 1995-484494 7 Jun 1995
PRIORITY INFO: US 1995-484494 7 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-480382 [41]
AB A new method for producing a **glycosylated iduronate**-2-**sulfatase** (I, EC-3.1.6.13) with a mol.wt. value of 65,000-95,000 involves culturing a host cell (e.g. **CHO**-KI or **CHO**-Lec1 cells) containing a DNA sequence encoding the protein, where the host cell **glycosylates** the protein to a greater degree than an **iduronate**-2-**sulfatase** (II) naturally expressed in human liver cells, and where the mol.wt. value of (I) is

5,000-40,000 more than (II). A DNA sequence isolated from human endothelial cells is disclosed. (I) deficiency in humans leads to the lysosomal accumulation of heparan sulfate and dermatan sulfate fragments and their excretion in urine. This storage causes Hunter syndrome (mucopolysaccharidosis type-II) in which patients may present variable phenotypes from severe mental retardation, skeletal deformities, and stiff joints, to a relatively mild course. (I) may be used to for diagnosis or therapy of (I)-associated diseases. (27pp)

L11 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:194978 HCAPLUS
DOCUMENT NUMBER: 128:261927
TITLE: **Glycosylation** variants of **iduronate** 2-**sulfatase**
INVENTOR(S): Wilson, Peter J.; Morris, Charles Phillip; Anson, Donald Stewart; Occhiodoro, Teresa; Bielicki, Julie; Clements, Peter Roy; Hopwood, John Joseph
PATENT ASSIGNEE(S): Australia
SOURCE: U.S., 53 pp. Division of U. S. Ser. No. 345,212.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5728381	A	19980317	US 1995-484493	19950607
US 5932211	A	19990803	US 1994-345212	19941128
US 5798239	A	19980825	US 1995-484494	19950607
PRIORITY APPLN. INFO.:			US 1991-790362	19911112
			US 1992-991973	19921217
			US 1994-345212	19941128

AB The present invention provides a highly **glycosylated** **iduronate**-2-**sulfatase** enzyme comprising an **iduronate**-2-**sulfatase** polypeptide with at least 5 kilodalton (kDa) more sugar than **iduronate**-2-**sulfatase** purified from a natural source, e.g. human liver. The present invention also provides an enzymically active polypeptide fragment or variant of such a highly **glycosylated** **iduronate**-2-**sulfatase**. The present invention further provides an isolated nucleic acid encoding **iduronate**-2-**sulfatase**, as well as an expression vector, a host cell and a method for producing the present highly **glycosylated** **iduronate**-2-**sulfatase** enzyme. In one embodiment the present invention is directed to a method for producing a **glycosylated** **iduronate**-2-**sulfatase** enzyme which comprises culturing a host cell contg. a nucleic acid encoding an enzymically active **iduronate**-2-**sulfatase** polypeptide wherein the host cell **glycosylates** the polypeptide to a greater degree than a native **iduronate**-2-**sulfatase** polypeptide expressed by a natural human liver cell.

L12 ANSWER 8 OF 11 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 95351969 MEDLINE
DOCUMENT NUMBER: 95351969
TITLE: Processing of iduronate 2-sulphatase in human fibroblasts.
AUTHOR: Froissart R; Millat G; Mathieu M; Bozon D; Maire I
CORPORATE SOURCE: Centre d'Etudes des Maladies Metaboliques, Hopital Debrousse, Lyon, France..
SOURCE: BIOCHEMICAL JOURNAL, (1995 Jul 15) 309 (Pt 2) 425-30.

Journal code: 9YO. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199511

AB Iduronate 2-sulphatase (**IDS**) is a lysosomal enzyme involved in degradation of dermatan sulphate and heparan sulphate. Antigenic material was obtained either by purification of placental **IDS** (A and B forms) or by expression of three different fusion peptides in Escherichia coli allowing the production of five specific antibodies. Pulse-chase-labelling experiments in over-expressing fibroblasts showed poor **IDS** processing but large amounts of precursors were secreted into the medium. The endocytosis of the 35S- or 33P-labelled precursors by deleted fibroblasts together with **glycosylation** studies and proteolysis inhibition by leupeptin allowed better elucidation of **IDS** maturation. The initial 73-78 kDa form is converted into a phosphorylated 90 kDa precursor after modification of its oligosaccharide chains in the Golgi apparatus. This precursor is processed by proteolytic cleavage through various intermediates to a major 55 kDa intermediate, with the release of an 18 kDa polypeptide. Further proteolytic cleavage by a thiol protease gives the 45 kDa mature form containing hybrid and complex-type oligosaccharide chains.

L12 ANSWER 1 OF 11 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1999:443869 BIOSIS
DOCUMENT NUMBER: PREV199900443869
TITLE: **Glycosylation** variants of **iduronate** 2-**sulfatase**.
AUTHOR(S): Wilson, Peter J. (1); Morris, Charles Phillip; Anson, Donald Stewart; Occhiodoro, Teresa; Bielicki, Julie; Clements, Peter Roy; Hopwood, John Joseph
CORPORATE SOURCE: (1) Women's and CChildren's Hosp, North Adelaide Australia
PATENT INFORMATION: US 5932211 Aug. 03, 1999
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 3, 1999) Vol. 1225, No. 1, pp. NO PAGINATION.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

L12 ANSWER 2 OF 11 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 1999268795 EMBASE
TITLE: [DSM-IV disorders, metabolic control and somatic complications in insulin-dependent diabetes mellitus of child and adolescent].
TROUBLES DSM-IV, EQUILIBRE METABOLIQUE ET COMPLICATIONS SOMATIQUES DANS LE DIABETE INSULINO-DEPENDANT DE L'ENFANT ET DE L'ADOLESCENT.
AUTHOR: Maronian S.; Vila G.; Robert J.-J.; Mouren-Simeoni M.-Ch.
CORPORATE SOURCE: G. Vila, Svc. Psychiat. l'Enfant/l'Adolescent, CHU Necker-Enfants Malades, 149, rue de Sevres, F-75015 Paris, France
SOURCE: Annales Medico-Psychologiques, (1999) 157/5 (320-331).
Refs: 40
ISSN: 0003-4487 CODEN: AMPYAT
COUNTRY: France
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
032 Psychiatry
LANGUAGE: French
SUMMARY LANGUAGE: English; French
AB Objectives: To describe the principal DSM-IV Mental Disorders, observed in a work of a paediatic liaison, in a population of insulin-dependent diabetic (IDDM) children and adolescents, and their association, according to the main diagnostic categories, with metabolic control and somatic complications; to study the relationship of familial environment socioeconomic status, age, sex of the patients and IDDM duration on metabolic control and somatic complications. Methods: We retrospectively included IDDM children and adolescents who have been systematically addressed in paedopsychiatry for three years for evaluation. They were 175 subjects, 96 girls and 79 boys, with a mean age of 13.5 .+-. 4.5 years and a mean IDDM duration of 4.9 .+-. 3.9 years. They were assessed by clinical interviews according with DSM-IV criteria. Metabolic control was measured by **glycosylated** haemoglobin (HBAIC which is a reflect of the glyacaemias of the last three months), at the first consultation in

paedopsychiatry and one year later. Somatic complications are systematically screened each year for all the IDDM patients (at least clinical examination, FO, retinian angiography, electroneurography and micro-albuminuria). Results: 102 patients (58.2%) had at least one DSM-IV disorder. The main disorders were anxiety disorders (33 subjects) and eating disorders (31 subjects); 24 subjects (13%) had affective disorders and 17 (9%) had disruptive behaviour disorders. Children and adolescents with mental disorders had a poorer metabolic control than others patients, at the first psychiatric consultation and one year later. Affective disorders, disruptive behaviour disorders and eating disorders were significantly associated with a poor metabolic control (measured by higher mean HBAIC), with a trend for patients with affective disorders and disruptive behaviour disorders to improve their HBAIC one year later. Patients with and without anxiety disorders had not significantly different HBAIC level. Familial problems, socioeconomic status and gender were not associated with HBAIC level; age and IDDM duration were correlated with HBAIC. Somatic complications (retinopathy and others) were associated with depressive disorders and an older age of the patients. Conclusions: This study shows that young IDDM patients did have DSM-IV disorders which were significantly associated with a poor metabolic control. So mental disorders should be systematically screened in children and adolescents with **IDS** and be treated to help to improve their metabolic control and their quality of life.

L12 ANSWER 3 OF 11 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-05849 BIOTECHDS
TITLE: Treatment of **iduronate-2-sulfatase** deficiency;
human recombinant enzyme preparation by expression in CHO-K1, or Lec-1 cell, used for e.g. Hunter disease therapy or gene therapy
AUTHOR: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J;
Clements P R; Hopwood J J
PATENT ASSIGNEE: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J;
Clements P R; Hopwood J J
LOCATION: North Adelaide, South Australia, Australia; Plympton, South
Australia, Australia; Thebarton, South Australia, Australia;
Prospect, South Australia, Australia.
PATENT INFO: US 5728381 17 Mar 1998
APPLICATION INFO: US 1995-484493 7 Jun 1995
PRIORITY INFO: US 1995-484493 7 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-206530 [18]
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L12 ANSWER 4 OF 11 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1998-[redacted]90 BIOTECHDS
TITLE: **Glyco[redacted]ation** variants of **iduronate**-2-**sulfatase**;
human recombinant enzyme preparation by vector-mediated
gene transfer and expression in host cell, used for Hunter
syndrome diagnosis or therapy
AUTHOR: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J;
Clements P R; Hopwood J J
PATENT ASSIGNEE: Women's+Child.Hosp.North-Adelaide
LOCATION: North Adelaide, South Australia, Australia.
PATENT INFO: US 5798239 25 Aug 1998
APPLICATION INFO: US 1995-484494 7 Jun 1995
PRIORITY INFO: US 1995-484494 7 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-480382 [41]

AB A new method for producing a **glycosylated iduronate**-2-**sulfatase** (I, EC-3.1.6.13) with a mol.wt. value of 65,000-95,000 involves culturing a host cell (e.g. CHO-KI or CHO-Lec1 cells) containing a DNA sequence encoding the protein, where the host cell **glycosylates** the protein to a greater degree than an **iduronate**-2-**sulfatase** (II) naturally expressed in human liver cells, and where the mol.wt. value of (I) is 5,000-40,000 more than (II). A DNA sequence isolated from human endothelial cells is disclosed.
(I) deficiency in humans leads to the lysosomal accumulation of heparan sulfate and dermatan sulfate fragments and their excretion in urine. This storage causes Hunter syndrome (mucopolysaccharidosis type-II) in which patients may present variable phenotypes from severe mental retardation, skeletal deformities, and stiff joints, to a relatively mild course. (I) may be used to for diagnosis or therapy of (I)-associated diseases. (27pp)

L12 ANSWER 5 OF 11 HCAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:194978 HCAPLUS
DOCUMENT NUMBER: 128:261927
TITLE: **Glycosylation** variants of **iduronate** 2-**sulfatase**
INVENTOR(S): Wilson, Peter J.; Morris, Charles Phillip; Anson, Donald Stewart; Occhiodoro, Teresa; Bielicki, Julie;
Clements, Peter Roy; Hopwood, John Joseph
PATENT ASSIGNEE(S): Australia
SOURCE: U.S., 53 pp. Division of U. S. Ser. No. 345,212.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5728381	A	19980317	US 1995-484493	19950607
US 5932211	A	19990803	US 1994-345212	19941128
US 5798239	A	19980825	US 1995-484494	19950607
PRIORITY APPLN. INFO.:			US 1991-790362	19911112
			US 1992-991973	19921217
			US 1994-345212	19941128

AB The present invention provides a highly **glycosylated** **iduronate**-2-**sulfatase** enzyme comprising an **iduronate**-2-**sulfatase** polypeptide with at least 5 kilodalton (kDa) more sugar than **iduronate**-2-**sulfatase** purified from a natural source, e.g. human liver. The present invention

also provides an enzymically active polypeptide fragment or variant of such a highly **glycosylated iduronate**-2-**sulfatase**. The present invention further provides an isolated nucleic acid encoding **iduronate**-2-**sulfatase**, as well as an expression vector, a host cell and a method for producing the present highly **glycosylated iduronate**-2-**sulfatase** enzyme. In one embodiment the present invention is directed to a method for producing a **glycosylated iduronate**-2-**sulfatase** enzyme which comprises culturing a host cell contg. a nucleic acid encoding an enzymically active **iduronate**-2-**sulfatase** polypeptide wherein the host cell **glycosylates** the polypeptide to a greater degree than a native **iduronate**-2-**sulfatase** polypeptide expressed by a natural human liver cell.

L12 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1997:100285 HCAPLUS
DOCUMENT NUMBER: 126:207933
TITLE: **IDS** transfer from overexpressing cells to **IDS**-deficient cells
AUTHOR(S): Millat, G.; Froissart, R.; Maire, I.; Bozon, D.
CORPORATE SOURCE: Centre d'etudes des Maladies Metaboliques, Hopital Debrousse, Lyon, 69322, Fr.
SOURCE: Exp. Cell Res. (1997), 230(2), 362-367
CODEN: ECREAL; ISSN: 0014-4827
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Iduronate sulfatase** (**IDS**) is responsible for mucopolysaccharidosis type II, a rare recessive X-linked lysosomal storage disease. The aim of this work was to test the ability of overexpressing cells to transfer **IDS** to deficient cells. In the first part of our work, **IDS** processing steps were compared in fibroblasts, COS cells, and lymphoblastoid cell lines and shown to be identical: the two precursor forms (76 and 90 kDa) were processed by a series of intermediate forms to the 55- and 45-kDa mature polypeptides. Then **IDS** transfer to **IDS**-deficient cells was tested either by incubation with cell-free medium of overexpressing cells or by coculture. Endocytosis and coculture expts. between transfected L.beta. and deleted fibroblasts showed that **IDS** transfer occurred preferentially by cell-to-cell contact as **IDS** precursors are poorly secreted by transfected L.beta.. The 76- and 62-kDa **IDS** polypeptides transferred to deleted fibroblasts were correctly processed to the mature 55- and 45-kDa forms. L.beta. were not able to internalize the 90-kDa phosphorylated precursor forms excreted in large amts. in the medium of overexpressing fibroblasts. Enzyme transfer occurred only by cell-to-cell contact, but the precursor forms transferred in L.beta. after cell-to-cell contact were not processed. This absence of maturation was probably due to a mistargeting of **IDS** precursors in these cells.

L12 ANSWER 7 OF 11 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 97479223 MEDLINE
DOCUMENT NUMBER: 97479223
TITLE: Characterization of iduronate sulphatase mutants affecting N-**glycosylation** sites and the cysteine-84 residue.
AUTHOR: Millat G; Froissart R; Maire I; Bozon D
CORPORATE SOURCE: Centre d'etudes des Maladies Metaboliques, Hopital Debrousse, Lyon, France.
SOURCE: BIOCHEMICAL JOURNAL, (1997 Aug 15) 326 (Pt 1) 243-7.

Journal code: 9YO. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199801
ENTRY WEEK: 19980104
AB Iduronate sulphatase (**IDS**) is responsible for mucopolysaccharidosis type II, a rare recessive X-linked lysosomal storage disease. The aim of this work was to evaluate the functional importance of each N-**glycosylation** site, and of the cysteine-84 residue. **IDS** mutant cDNAs, lacking one of the eight potential N-**glycosylation** sites, were expressed in COS cells. Although each of the potential sites was used, none of the eight **glycosylation** sites appeared to be essential for lysosomal targeting. Another important sulphatase co- or post-translational modification for generating catalytic activity involves the conversion of a cysteine residue surrounded by a conserved sequence C-X-P-S-R into a 2-amino-3-oxopropionic acid residue [Schmidt, Selmer, Ingendoh and von Figura (1995) Cell 82, 271-278]. This conserved cysteine, located at amino acid position 84 in **IDS**, was replaced either by an alanine (C84A) or by a threonine (C84T) using site-directed mutagenesis. C84A and C84T mutant cDNAs were expressed either in COS cells or in human lymphoblastoid cells deleted for the **IDS** gene. C84A had a drastic effect both for **IDS** processing and for catalytic activity. The C84T mutation produced a small amount of mature forms but also abolished enzyme activity, confirming that the cysteine residue at position 84 is required for **IDS** activity.

L12 ANSWER 8 OF 11 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 95351969 MEDLINE
DOCUMENT NUMBER: 95351969
TITLE: Processing of iduronate 2-sulphatase in human fibroblasts.
AUTHOR: Froissart R; Millat G; Mathieu M; Bozon D; Maire I
CORPORATE SOURCE: Centre d'Etudes des Maladies Metaboliques, Hopital Debrousse, Lyon, France..
SOURCE: BIOCHEMICAL JOURNAL, (1995 Jul 15) 309 (Pt 2) 425-30.

Journal code: 9YO. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199511
AB Iduronate 2-sulphatase (**IDS**) is a lysosomal enzyme involved in degradation of dermatan sulphate and heparan sulphate. Antigenic material was obtained either by purification of placental **IDS** (A and B forms) or by expression of three different fusion peptides in Escherichia coli allowing the production of five specific antibodies. Pulse-chase-labelling experiments in over-expressing fibroblasts showed poor **IDS** processing but large amounts of precursors were secreted into the medium. The endocytosis of the 35S- or 33P-labelled precursors by deleted fibroblasts together with **glycosylation** studies and proteolysis inhibition by leupeptin allowed better elucidation of **IDS** maturation. The initial 73-78 kDa form is converted into a phosphorylated 90 kDa precursor after modification of its oligosaccharide chains in the Golgi apparatus. This precursor is processed

by proteolytic cleavage through various intermediates to a major 55 kDa intermediate, with the release of an 18 kDa polypeptide. Further proteolytic cleavage by a thiol protease gives the 45 kDa mature form containing hybrid and complex-type oligosaccharide chains.

L12 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1995:694170 HCAPLUS
DOCUMENT NUMBER: 123:109674
TITLE: FIV vaccine studies. I. Immune response to recombinant FIV env gene products and outcome after challenge infection
AUTHOR(S): Lutz, H.; Hofmann-Lehmann, R.; Bauer-Pham, K.; Holznagel, E.; Tozzini, F.; Bendinelli, M.; Reubel, G.; Aubert, A.; Davis, D.; et al.
CORPORATE SOURCE: Department Internal Veterinary Medicine, University Zurich, Zurich, Switz.
SOURCE: Vet. Immunol. Immunopathol. (1995), 46(1,2), 103-13
CODEN: VIIMDS; ISSN: 0165-2427
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors vaccinated five groups of cats (n = 25) four times with five prepns. of recombinant feline immunodeficiency virus (FIV) env gene products; one group (n = 7) served as control. The vaccine formulations were as follows: (1) envelope glycoprotein of FIV Zurich 2 (FIV Z2) expressed in a Baculovirus system and isolated by gel electroelution (denatured form); (2) insect cells expressing FIV Z2 glycoprotein; (3) envelope glycoprotein of a Boston strain (FIV Bangston) expressed in insect cells and isolated by gel electroelution (denatured form); (4) **glycosylated** Bangston envelope protein made in insect cells and isolated in a native form; (5) non-**glycosylated** Bangston envelope protein made in Escherichia coli. All cats were challenged with 20 50% cat **IDs** (CID50) of FIV Z2 previously titrated in cats. All vaccinated cats developed high ELISA antibodies to the homologous antigen; crossreactivity to heterologous antigens was seen at a lower level. Virus neutralizing antibodies (tested with Petaluma virus) reached titers up to 32. After challenge, all cats seroconverted (as judged by anti gag antibodies in Western blot) and became infected (as judged by virus isolation and/or polymerase chain reaction) between 4 and 11 wk with the exception of one cat. It is concluded that it is relatively easy to induce high ELISA antibody titers using recombinant env gene products, ELISA antibody titers do not correlate with virus neutralization or with protection.

L12 ANSWER 10 OF 11 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 92095973 MEDLINE
DOCUMENT NUMBER: 92095973
TITLE: Morquio disease: isolation, characterization and expression of full-length cDNA for human N-acetylgalactosamine-6-sulfate sulfatase.
AUTHOR: Tomatsu S; Fukuda S; Masue M; Sukegawa K; Fukao T; Yamagishi A; Hori T; Iwata H; Ogawa T; Nakashima Y; et al
CORPORATE SOURCE: Department of Pediatrics, Gifu University School of Medicine, Japan.
SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1991 Dec 16) 181 (2) 677-83.

Journal code: 9Y8. ISSN: 0006-291X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
OTHER SOURCE: GENBANK-S70932; GENBANK-S70919; GENBANK-S74367; GENBANK-S67100; GENBANK-M64055; GENBANK-M64056; GENBANK-M64057; GENBANK-M64058; GENBANK-M64059; GENBANK-M64060
ENTRY MONTH: 199204

AB We cloned and sequenced a full-length cDNA of human placental N-acetylgalactosamine-6-sulfate sulfatase, the enzyme deficient in Morquio disease. The 2339-nucleotide sequence contained 1566 nucleotides which encoded a polypeptide of 522 amino acid residues. The deduced amino acid sequence was composed of a 26-amino acid N-terminal signal peptide and a mature polypeptide of 496 amino acid residues including two potential asparagine-linked **glycosylation** sites. Expression of the cDNA in transfected deficient fibroblasts resulted in higher production of this sulfatase activity than in untransfected deficient fibroblasts. The cDNA clone was hybridized to only a 2.3-kilobase species of RNA in human fibroblasts. The amino acid sequence of N-acetylgalactosamine-6-sulfate sulfatase showed a high degree of homology with those of other sulfatases such as human arylsulfatases A, B or C, glucosamine-6-sulfatase, **iduronate**-2-**sulfatase** and sea urchin arylsulfatase.

L12 ANSWER 11 OF 11 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 81163947 MEDLINE
DOCUMENT NUMBER: 81163947
TITLE: Long-term, ambulatory, subcutaneous insulin infusion versus multiple daily injections in brittle diabetic patients.
AUTHOR: Barbosa J; Menth L; Eaton J; Sutherland D; Freier E F; Najarian J
CONTRACT NUMBER: AM-20742 (NIADDK)
2-P01-AM13083 (NIADDK)
RR-400 (NCRR)
SOURCE: DIABETES CARE, (1981 Mar-Apr) 4 (2) 269-74.
Journal code: EAG. ISSN: 0149-5992.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198108

AB We compared the blood glucose control of four intact and eight kidney recipient, metabolically unstable, ketosis-prone, insulin-dependent diabetic patients under two different regimens: (a) intensive conventional treatment with two to four insulin injections daily (48 patient-months) and (B) subcutaneous, portable insulin delivery system (**IDS**) (54 patient-months). Both regimens included frequent home blood glucose and 24-h urine glucose determinations and daily telephone follow-up to maximize compliance with treatment. Analyzed as a group the fasting blood glucose for intact patients (A: 172 +/- 13 mg/dl; B: 141 +/- 12, P less than 0.02) and the nonfasting blood glucose for kidney recipient patients (A: 165 +/- 10; B: 138 +/- 5, P less than 0.01) were significantly lower during treatment with the **IDS** than with multiple injections. Six out of 12 patients (2/4 intact and 4/8 kidney recipient patients) showed

significant and consistent improvement of blood glucose concentrations.

Four showed marginal and inconsistent improvement. Two patients (one intact and one kidney recipient) improved on the **IDS** but maintained the improvement when changed back to conventional treatment.

The 24-h urine glucose, maximal glucose excursions, number of blood glucoses less than or equal to 40 mg/dl, and **glycosylated** hemoglobin decreased significantly in some patients on the pump. We conclude that subcutaneous, portable insulin delivery devices can significantly improve the metabolic control of some ambulatory, unstable diabetic patients during long-term treatment beyond that obtained with intensive, multiple-injection, conventional treatment. Normalization of the metabolic control, however, is not obtained. These infusion systems still pose several problems during ambulatory use, which could have serious consequences in patients less compliant and/or followed less closely than ours.

=> d 1-5 ibib ab

L16 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-05849 BIOTECHDS
TITLE: Treatment of iduronate-2-sulfatase deficiency; human recombinant enzyme preparation by expression in CHO-K1, or Lec-1 cell, used for e.g. Hunter disease therapy or gene therapy
AUTHOR: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J; Clements P R; Hopwood J J
PATENT ASSIGNEE: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J; Clements P R; Hopwood J J
LOCATION: North Adelaide, South Australia, Australia; Plympton, South Australia, Australia; Thebarton, South Australia, Australia; Prospect, South Australia, Australia.
PATENT INFO: US 5728381 17 Mar 1998
APPLICATION INFO: US 1995-484493 7 Jun 1995
PRIORITY INFO: US 1995-484493 7 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-206530 [18]
AB A new method for treating iduronate-2-sulfatase (IDS, EC-3.1.16.13) deficiency, e.g. Hunter disease, involves administering a human **recombinant IDS** that is more highly glycosylated than the naturally occurring enzyme. The **recombinant IDS** has better uptake properties and/or a longer half-life in vivo and is therefore more efficacious than naturally glycosylated IDS. Also disclosed are a human **recombinant IDS** with a defined 550 amino acid protein sequence and an isolated genomic DNA fragment carrying all or part of the IDS gene. Isolation of the genomic clone may enable gene therapy and genetic analysis of IDS deficiency diseases. Preferably, the **recombinant IDS** has a mol.wt. value of 70,000-90,000 by SDS-PAGE and is produced in CHO-K1 or Lec-1 cells.
(53pp)

L16 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1998-10390 BIOTECHDS
TITLE: Glycosylation variants of iduronate-2-sulfatase; human recombinant enzyme preparation by vector-mediated gene transfer and expression in host cell, used for Hunter syndrome diagnosis or therapy
AUTHOR: Wilson P J; Morris C P; Anson D S; Occhiodoro T; Bielicki J; Clements P R; Hopwood J J
PATENT ASSIGNEE: Women's+Child.Hosp.North-Adelaide
LOCATION: North Adelaide, South Australia, Australia.
PATENT INFO: US 5798239 25 Aug 1998
APPLICATION INFO: US 1995-484494 7 Jun 1995
PRIORITY INFO: US 1995-484494 7 Jun 1995
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-480382 [41]
AB A new method for producing a glycosylated iduronate-2-sulfatase (I, EC-3.1.6.13) with a mol.wt. value of 65,000-95,000 involves culturing a

host cell (e.g. CHO-KI or CHO-Lec1 cells) containing a DNA sequence encoding the protein, where the host cell glycosylates the protein to a greater degree than an iduronate-2-sulfatase (II) naturally expressed in human liver cells, and where the mol.wt. value of (I) is 5,000-40,000 more than (II). A DNA sequence isolated from human endothelial cells is disclosed. (I) deficiency in humans leads to the lysosomal accumulation of heparan sulfate and dermatan sulfate fragments and their excretion in urine. This storage causes Hunter syndrome (mucopolysaccharidosis type-II) in which patients may present variable phenotypes from severe mental retardation, skeletal deformities, and stiff joints, to a relatively mild course. (I) may be used to for diagnosis or therapy of (I)-associated diseases. (27pp)

L16 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1994-13487 BIOTECHDS
TITLE: Pre-clinical studies of lymphocyte gene therapy for Hunter syndrome;
human peripheral blood lymphocyte transduction with LSXN-derived retro virus vector for iduronate-sulfatase gene expression (conference abstract)
AUTHOR: Pan D; Braun S E; Jonsson J J; Aronovich E L; McIvor R S; Whitley C B
CORPORATE SOURCE: Univ.Minnesota-Med.Sch.
LOCATION: Gene Therapy Program, University of Minnesota Medical School, Minneapolis, MN 55455, USA.
SOURCE: J.Cell.Biochem.; (1994) Suppl.18A, 244
CODEN: JCEBD5
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Retro virus constructs were studied and included: L2SN and LNC2 (derived from LXSN) with the iduronate-sulfatase (IDS) gene under the control of the long terminal repeat or cytomegalo virus promoter; L2 with no selectable marker; and LB2 utilizing the beta-actin promoter. Following 3.5 days of T-lymphocyte stimulation (OKT3, interleukin-2), peripheral blood lymphocytes were transduced on 4 consecutive days with PA317 supernatant in the presence of protamine sulfate. After culture for 3-6 days without G418 selection, peripheral blood lymphocytes were assayed for IDS activity, tested for 35SO4-GAG accumulation versus time and co-cultured for 2 days to assess 35SO4-GAG accumulation in neighboring fibroblasts from patients with Hunter syndrome. IDS activity was increased in transduced cells above peripheral blood lymphocyte LXSN controls or leukocyte levels of patients and comparable to white blood cell levels of normal individuals. The transduced cells failed to show continued accumulation of 35SO4-GAG, showing that **recombinant IDS** corrected the metabolic defect. Mild, non-neuropathic Hunter syndrome is suitable for ex vivo lymphocyte gene therapy. (0 ref)

L16 ANSWER 4 OF 5 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 94089725 MEDLINE
DOCUMENT NUMBER: 94089725
TITLE: Metabolic correction and cross-correction of mucopolysaccharidosis type II (Hunter syndrome) by

retroviral-mediated gene transfer and expression of human
iduronate-2-sulfatase.
AUTHOR: Braun S E; Aronovich E L; Anderson R A; Crotty P L; McIvor
R S; Whitley C B
CORPORATE SOURCE: Department of Genetics and Cell Biology, University of Minnesota, Minneapolis 55455..
CONTRACT NUMBER: RO1DK39891 (NIDDK)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Dec 15) 90 (24) 11830-4.
Journal code: PV3. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199403
AB To explore the possibility of using gene transfer to provide iduronate-2-sulfatase (IDS; EC 3.1.6.13) enzyme activity for treatment of Hunter syndrome, an amphotropic retroviral vector, L2SN, containing the human IDS coding sequence was constructed and studied for gene expression in vitro. Lymphoblastoid cell lines (LCLs) from patients with Hunter syndrome were transduced with L2SN and expressed high levels of IDS enzyme activity, 10- to 70-fold higher than normal human peripheral blood leukocytes or LCLs. Such L2SN-transduced LCLs failed to show accumulation of 35SO4 into glycosaminoglycan (35SO4-GAG), indicating that **recombinant IDS** enzyme participated in GAG metabolism. Coculture of L2SN-transduced LCLs with fibroblasts from patients with Hunter syndrome reduced the accumulation of 35SO4-GAG. These results demonstrated retroviral-mediated IDS gene transfer into lymphoid cells and the ability of such cells to provide recombinant enzyme for intercellular metabolic cross-correction.

L16 ANSWER 5 OF 5 BIOTECHDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1993-02607 BIOTECHDS
TITLE: Recombinant human iduronate-2-sulfatase: correction of mucopolysaccharidosis-type-II fibroblasts and characterization of the purified enzyme; gene cloning and expression in CHO-K1 cell culture for potential use in enzyme replacement therapy
AUTHOR: Bielicki J; Hopwood J J; Wilson P J; *Anson D S
LOCATION: Lysosomal Diseases Research Unit, Department of Chemical Pathology, Adelaide Children's Hospital, 72 King William Road, North Adelaide, South Australia 5006, Australia.
SOURCE: Biochem.J.; (1993) 289, Pt.1, 241-46
CODEN: BIJOAK
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In order to evaluate enzyme replacement therapy for mucopolysaccharidosis type-II, a chimeric iduronate-2-sulfatase (I2S, EC-3.1.6.13) cDNA was cloned and expressed in a CHO-K1 cell culture, using a vector (plasmid pB12Sc17) which placed the cDNA under the transcriptional control of the human polypeptide chain elongation factor-1-alpha gene promoter. A cell line that accumulated recombinant I2S at more than 10 mg/ml in conditioned medium was identified. Cells were grown to confluency in serum-free culture medium in two-layer cell factories, and recombinant I2S was purified to homogeneity by PBE94 and Blue-A-agarose chromatography, and by FPLC, resulting in purification of mg quantities

of recombinant I2S. The enzyme had a pH optimum and kinetic parameters
similar to those for the mature form of I2S purified from human liver.
The recombinant I2S had a mol.wt. of 90,000, which was reduced to 60,000
by deglycosylation using endoglycosidase. (11 ref)